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Effect of pentobarbital and isoflurane on acute stress response in rat

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HIGHLIGHTS

- Anesthesia before sacrificing animals may interfere with stress research.
- I.p. pentobarbital and isoflurane inhalation do not affect brain mRNA levels.
- I.p. injection causes extra stress and interferes with plasma corticosterone.
- Isoflurane inhalation leaves the stress response intact and is ethically optimal.

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ABSTRACT

Background: Anesthesia administration before sacrificing animals is a common practice in stress-related studies, but the effect of anesthesia on the results remains understudied. We aimed to reveal the interference of different anesthetics, i.e. intraperitoneal (i.p.) sodium-pentobarbital injection or isoflurane inhalation, with the acute stress responses in rats.

Methods: Rats were randomly divided into foot shock (FS) and non-stressed control groups, and further grouped according to the sacrificing procedure: direct decapitation, decapitation after i.p. sodium-pentobarbital injection, or isoflurane inhalation. There was also a non-stressed group sacrificed by decapitation following i.p. saline injection. Plasma levels of corticosterone (CORT), testosterone and estradiol, hypothalamic stress-related molecule mRNA expression of corticotropin-releasing hormone, arginine vasopressin and oxytocin, and frontal lobe stress-related molecule mRNA expression of NMDA receptor subunit NR2B, GABAA receptor and the neuronal-type nicotinic acetylcholine receptor were measured.

Results: FS significantly increased plasma CORT levels in direct decapitation and isoflurane groups, while this stress response 'disappeared' following i.p. sodium-pentobarbital injection. In control animals, both the injection of saline and pentobarbital caused a significant increase of plasma CORT. Neither the sex hormone levels nor the mRNA expression of stress-related molecules in the brain showed significant differences among the groups.

Conclusion: The injection of the anesthetic compound rather than the compound itself may cause extra stress which interferes with the plasma CORT levels, but not with plasma sex hormone levels nor with the brain mRNA expression. Isoflurane inhalation leaves the stress response intact and is also optimal from an ethical point of view.

1. Introduction

The relationship between disordered stress responses and the etiology of affective disorders [1] has boosted studies on the stress responses in animal models. Activities of stress-regulating systems such as the hypothalamo-pituitary-adrenal (HPA) axis have been studied in different stressful events. HPA activity is driven by the corticotropin-releasing hormone (CRH) which is mainly secreted from the paraventricular nucleus (PVN) of the hypothalamus. The end-target stress hormones of the HPA axis are corticosteroids, which in rats is mainly corticosterone

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(CORT). We and other groups have observed that sex steroids such as testosterone (T) and estradiol (E2), and neuropeptides such as arginine vasopressin (AVP) and oxytocin (OXT), play a crucial role in the regulation of the stress response [2].

The possible confounding effect of the way of sacrificing animals for research has raised our concerns. There are in general 2 ways of sacrificing animals for stress-related biological studies: direct decapitation or decapitation following administration of an anesthetic. Decapitation may be a potential extra stressor, which also depends on the experience of the researcher involved, and may interfere with the study results [3]. Anesthetics that induce unconsciousness seem to diminish such extra stress [4]. However, some anesthetics, such as pentobarbital, have been found to induce an extra stress response, manifested as increased plasma CORT levels [5,6]. In addition, anesthetics affect brain activity by inhibition of excitatory receptors, including N-methyl-D-aspartate (NMDA) receptor subunit NR2B and the neuronal-type nicotinic acetylcholine receptor (nnAChR), and by stimulation of inhibitory receptors such as GABAA receptor (GABAAR) [7]. So far it is unclear whether the often used anesthetics such as intraperitoneal (i.p.) injection of sodium-pentobarbital or isoflurane inhalation may affect rat brain mRNA expression of stress-related molecules in response to acute stress, neither is it clear how i.p. injection of sodium-pentobarbital causes extra stress to animals.

We therefore analyzed the effect of different ways of sacrificing animals, i.e. decapitation by an experienced researcher, decapitation following i.p. injection of sodium-pentobarbital and decapitation following isoflurane inhalation in rats that experienced electric foot shocks (FS) stress and compared them to non-stressed controls. We determined the changes in stress-related molecules, including plasma CORT, T and E2 levels, the hypothalamic mRNA levels of CRH, AVP and OXT, and the frontal lobe mRNA levels of NMDA-NR2B, GABAAR and nnAChR.

2. Methods

2.1. Ethics statement

All animal care and all procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996, USA) approved by the National Committee to the Use of Experimental Animals for Medical Purposes, China, Zhejiang Branch. Every effort was made to minimize animal suffering and to use as few animals as possible (for more details see Supplementary Material 1).

2.2. FS rats

Male Sprague Dawley (SD) rats (body weight 300–330 g) were housed in an air-conditioned room at 25–27 °C, in a 12:12-hour light–dark cycle, and given food and water ad libitum. Prior to the experiments the rats were allowed 1 week to adapt to the environmental conditions. The protocol of FS was 0.5 mA during 5 s, followed by 25 s blank for 20 min [8,9]. For details of animal housing conditions and health, together with the FS protocol see Supplementary Material 1. The rats were randomly divided into 7 groups: 1) control or 2) FS rats sacrificed both by direct decapitation (CTRD and FSD respectively); 3) control or 4) FS rats both sacrificed by decapitation 5 min after i.p. injection of 3.5% sodium-pentobarbital (3 ml/kg) (CTRP and FSP respectively); 5) control or 6) FS rats both sacrificed by decapitation 1–2 min after inhalation of 4% isoflurane in oxygen (CTRI or FSI group); and 7) an extra control group sacrificed by decapitation 5 min after i.p. injection of normal saline (3 ml/kg) (CTRS). Each group contained 7 or 8 rats. The animals were handled daily and sacrificed between 15:00 hours and 16:00 hours, within 30 min from the onset of FS, always by the same researcher. Trunk blood was collected, centrifuged and plasma was collected. The rat brain was rapidly removed and the hypothalamus and the frontal lobe were dissected. The hypothalamus was dissected along the following borders: anterior of the optic chiasm, posterior of the mamillary bodies, and lateral, the hypothalamic sulci. The dorsal cut was at approximately 3 mm from the bottom. For dissection of the frontal lobe, the olfactory lobe was first removed, and then a frontal

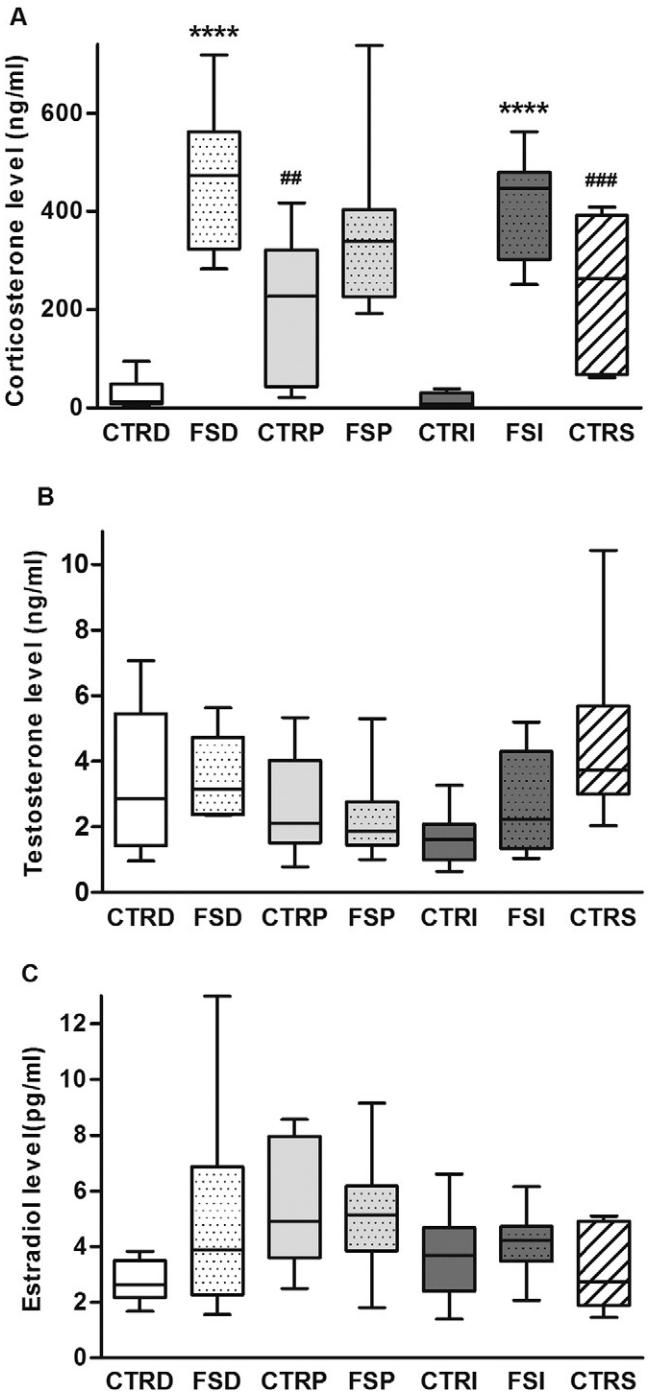

Fig. 1. The effects of intraperitoneal (i.p.) injection of sodium-pentobarbital or isoflurane inhalation anesthesia on the stress response as reflected in plasma hormone levels. FS: foot shock; CTRD or FSD: control or FS rats sacrificed by direct decapitation (n = 8/group); CTRP or FSP: control or FS rats sacrificed by decapitation 5 min after i.p. injection of 3.5% sodium-pentobarbital (3 ml/kg, n = 8/group); CTRI or FSI: control or FS rats sacrificed by decapitation 1–2 min after inhalation of 4% isoflurane in oxygen (n = 8/group); CTRS: control group sacrificed by decapitation 5 min after i.p. injection of normal saline (3 ml/kg, n = 7/group). Data are shown as median, 25th–75th percentiles, and the range. $^{****}P < 0.001$: FSD compared with CTRD; and FSI compared with CTRI; $^{\#\#}P < 0.01$: CTRP compared with CTRD; $^{\#\#\#}P < 0.005$: CTRS compared with CTRD.